

# DIMORPHIC INCOMPATIBILITY IN *TURNERA HERMANNIOIDES* CAMB. (TURNERACEAE)<sup>1</sup>

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## ABSTRACT

*Turnera hermannioides* (Turneraceae) is a distylous, diploid, perennial restricted to open, sandy sites in northeastern Brazil. Four populations sampled in Sergipé contained equal proportions of long- and short-styled plants. The style and stamen dimorphism in floral morphs is associated with differences in pollen size and pollen production. In contrast, flower size, ovule number, and seed fertility of the morphs are not significantly different. A controlled pollination program demonstrated that individuals of *T. hermannioides* possess a self- and intra-morph incompatibility system. The only pollinations resulting in significant seed production were between the floral morphs. Populations of *T. hermannioides* grow intermingled with *T. ulmifolia*, a widespread polymorphic complex containing forms that resemble *T. hermannioides*. A crossing program between *T. hermannioides* and diploid and tetraploid varieties of *T. ulmifolia* revealed barriers to hybridization particularly in crosses at the diploid level. It is concluded that *T. hermannioides* is a restricted diploid species that is reproductively isolated from *T. ulmifolia*.

Distyly is reliably reported from 23 flowering plant families (Ganders, 1979). In taxa on which experimental work has been undertaken, it has usually been shown that the stamen-style dimorphism is closely associated with a diallelic self-incompatibility system as well as several morphological polymorphisms involving pollen size, pollen production, and stigmatic papillae number and length. Although there is general agreement that distyly is an outbreeding mechanism (Darwin, 1877; Crowe, 1964; Vuilleumier, 1967), the functions of the various floral polymorphisms that accompany self-incompatibility are still unclear (Yeo, 1975; Ganders, 1979).

The large neotropical genus *Turnera* (Turneraceae) contains some 54 species of which 37 are reported to be heterostylous (Urban, 1883). Species within the genus are poorly defined, and there is need for a modern taxonomic revision of the group. With the exception of studies of distyly in the widespread polymorphic weed complex *T. ulmifolia* L. (including the synonyms *T. subulata* Smith and *T. trioniflora* Sims) by Lock (1904), Barrett (1978), and Bentley (1979), there are no published reports of experimental work on the reproductive biology of species of *Turnera*. To broaden understanding of the na-

ture of distyly in the genus, we initiated a study of the breeding system of *T. hermannioides*, a herbaceous to woody perennial native to northeastern Brazil. In addition to documenting general features of distyly, incompatibility relationships, and population structure in the species, we also investigated its crossing relationships with *T. ulmifolia*, with which it is superficially similar in habit (Urban, 1883) and frequently sympatric.

## MATERIALS AND METHODS

All populations of *Turnera hermannioides* studied occur in the state of Sergipé in northeastern Brazil. Detailed localities are presented in Table 1. At each population, the number of long- and short-styled plants was recorded. In populations 2, 3, and 4, the entire flowering population was scored whereas in population 1, a complete sample of the accessible part of the population (ca. one-third of the total size) was made (Table 5). Deviations from the expected 1:1 morph frequency were analyzed using the G-statistic which was calculated for each population. In addition, pooled and heterogeneity G-statistics were calculated (Sokal & Rohlf, 1981) to examine whether population structures were similar.

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TABLE 1. Localities of *Turnera hermannioides* and *Turnera ulmifolia* populations used in this study. All populations of *T. hermannioides* were collected along roadsides on open, waste ground.

A. <i>Turnera hermannioides</i>		
Population	Locality	Varieties of <i>T. ulmifolia</i> Present at Site
1 <sup>a</sup>	Hwy. 235, ca. 30 km W of Itabaiana, Sergipé	<i>elegans</i>
2	Hwy. 235, ca. 15 km W of Itabaiana, Sergipé	<i>elegans</i>
3	Hwy. 235, ca. 1 km W of Itabaiana, Sergipé	<i>elegans</i>
4 <sup>b</sup>	Hwy. 235, 21 km S of BR101, near Aracaju, Sergipé	<i>elegans</i> and <i>surinamensis</i>

B. *Turnera ulmifolia*

Variety	Locality	Chromosome Number and Ploidy Level <sup>c</sup>
<i>surinamensis</i>	Santarém, Pará, Brazil	$2n = 2x = 10$
<i>grandiflora</i>	Corrientes, Argentina	$2n = 2x = 10$
<i>intermedia</i>	Barreirinhas, Maranhão, Brazil	$2n = 2x = 10$
<i>intermedia</i>	St. Cristobal, Dominican Republic	$2n = 4x = 20$
<i>elegans</i>	Crato, Ceara, Brazil	$2n = 4x = 20$

<sup>a</sup> Vouchers for chromosome counts, Shore and Barrett 1 TRT.

<sup>b</sup> Vouchers for chromosome counts, Shore and Barrett 2 TRT.

<sup>c</sup> Vouchers for chromosome counts, Shore and Barrett (unpubl. data).

At population 1, ten flowers of each style form were collected and the style length, stamen length, and corolla diameter were measured with calipers to the nearest 0.1 mm (Table 2). Style and stamen lengths were measured from the base of the ovary to the top of the male or female reproductive organ. Flower buds, 2–4 mm in length, were collected and fixed in 3:1 ethanol:acetic acid for chromosome number determination. Later, anther squashes were prepared in 2% aceto-carmin, and meiosis was observed in pollen mother cells. Eighteen larger buds were collected separately from 18 individuals of each style morph to determine pollen production. Pollen counts were made using the hemacytometer method of Lloyd (1965). Anthers from three buds per morph were pooled for each value obtained. Six replicate samples were then determined for each morph. Data were analyzed by nested ANOVA.

A bulk seed sample was collected at population 4 where ample ripe seed was available. The seed was sown in the summer of 1982 in a soil mixture of 3 parts sterilized potting compost (1 peat:1 clay-loam:1 sand):1 sand:1 peat, in 7.5 cm plastic pots. Seeds were covered with about 1 cm soil and allowed to imbibe water for one week. As the soil was allowed to dry, germination proceeded. Nine short-styled and 13 long-styled

plants were obtained. When they had reached the 5-leaved stage, these were then transplanted into 12 cm clay pots and grown under uniform glasshouse conditions in air temperatures ranging from 25°–35°C. The plants were the material used for experimental work described below.

The polar and equatorial axes of five pollen grains were measured for each of seven individuals per morph. Pollen grains were measured to the nearest 1.5  $\mu\text{m}$  using a calibrated ocular micrometer on a compound microscope. Nested ANOVA was used to examine variation among individuals and between style morphs.

A controlled crossing program was initiated in May 1983, to determine the compatibility relationships of the floral morphs (Table 4). Each individual was self-pollinated at least twice, crossed to at least two different individuals of the same floral morph, and to a minimum of two individuals of the opposite floral morph. Pollinations were performed in a pollinator-free glasshouse. Pollen was transferred from the male to the female parent by removing all stamens with a pair of clean, fine forceps and rubbing the dehiscent anther sacs on recipient stigmas. The number of seeds set per pollination was recorded. For inter-morph crosses, capsules were harvested prior to dehiscence, which normally occurred approximately 24 days after pollination, and dis-



TABLE 2. Floral characters in the long- and short-styled morphs of *Turnera hermannioides*. Values are the mean and standard deviation. Measurements of floral organs and pollen production were made from field grown plants in population 1, the remaining characters were from glasshouse grown plants originating from seed collected in population 4. NS = not significant.

Character	Floral Morph		F	P
	Long-styled	Short-styled		
Flower diameter (mm)	33.9 ± 2.8	34 ± 5.1	0.007	NS
Style length (mm)	12 ± 1.1	7.3 ± 0.9	114.0	<0.001
Stamen length (mm)	7.3 ± 0.6	12 ± 0.8	221.0	<0.001
Pollen size (μm) (polar axis)	63.6 ± 2	72.9 ± 2.6	61.7	<0.001
Pollen size (μm) (equatorial axis)	33 ± 0.9	39.6 ± 1.5	117.0	<0.001
Pollen production <sup>-flower</sup>	15,200 ± 2,700	11,800 ± 2,200	6.14	<0.05
Ovule number <sup>-flower</sup>	32.3 ± 9.9	39 ± 8.8	2.4	NS
Seeds <sup>-capsule a</sup>	20 ± 10.1	18.9 ± 10.1	0.14	NS

<sup>a</sup> Following inter-morph pollination.

sected open. This procedure enables determination of the number of seeds set and the total number of ovules produced in a flower. Data on seed set and ovule number were analyzed by nested ANOVA using a sample of nine individuals of each morph and two replicate measures for each individual.

A second crossing program was initiated in June 1983, to determine the crossing relationships of *T. hermannioides* with taxa in the related *T. ulmifolia* complex (Table 6). Five population samples of four varieties were used. Five individuals from each population, including *T. hermannioides* were used. The varietal status, locality, and ploidal level of members of the *T. ulmifolia* complex are provided in Table 1. Further details of the reproductive biology of populations are presented in Barrett (1978). Pollinations were performed as described above. Capsules were harvested after dehiscence. Seeds were retained in the capsules by placing parafilm over the swollen ovary about ten days after pollination. For each cross the number of brown plump seeds produced per pollination was recorded.

## RESULTS

**Floral morphology.** Flowers of *Turnera hermannioides* exhibit a precise reciprocity in the lengths of stamens and styles between the long- and short-styled morphs (Table 2). There was no significant difference between the floral morphs in flower diameter, number of ovules per flower, and seed set following legitimate (intermorph) pollination. In common with many distylous plants, *T. hermannioides* exhibited dimorphism

in the size of pollen grains. Both axes of pollen grains are longer in the short-styled morph. Pollen production per flower in the long-styled morph was significantly higher than in the short-styled morph (Table 2). Microscopic observation of stigmas of the floral morphs revealed the absence of stigmatic papillae, a feature shared with *T. ulmifolia*, and in contrast to most heterostylous species.

Where appropriate data were available, nested ANOVAs were undertaken on reproductive traits in order to partition the observed variation into the three possible sources (among morphs, among individuals, and error). In our samples, significant variation occurred in ovule number per flower among individuals, but not between the floral morphs (Table 3). Large samples clearly would be required to determine if morph-specific differences in ovule number occur. The data for ovule number and pollen production suggest that

TABLE 3. Variance components of reproductive characters in *Turnera hermannioides*. Values are the percentage of the observed variation which is attributable to individual variation, floral morph, and error following nested analysis of variance.

Character	Individual	Morph	Residual
Ovule number	70 <sup>b</sup>	13	17
Pollen production	40 <sup>b</sup>	38 <sup>a</sup>	22
Pollen size (polar axis)	9 <sup>b</sup>	85 <sup>b</sup>	6

<sup>a</sup>  $P < 0.05$ .

<sup>b</sup>  $P < 0.001$ .



TABLE 4. Fruit and seed set following controlled self, intra-morph and inter-morph cross-pollination of the long- and short-styled morphs of *Turnera hermannioides*. Plants were grown from seed collected in population 4; all pollinations were performed under glasshouse conditions.

Pollination Treatment	No. of ♀ Plants	No. of Flowers Pollinated	% Fruit Set	No. of Seeds Produced	Mean Seed Set per Capsule ± s.d.
L self	13	87	10	13	1.4 ± 0.7
S self	9	62	13	10	1.3 ± 0.7
L × L	13	28	25	7	2.3 ± 0.3
S × S	9	18	6	1	1
L × S	13	27	100	540	20 ± 10.1
S × L	9	18	100	341	18.9 ± 10.1

considerable individual variation in fertility may exist within populations of *T. hermannioides*.

*Compatibility relationships.* The controlled pollination program demonstrated the presence of a physiological self-incompatibility system in *T. hermannioides*. Self- and intra-morph pollinations resulted in relatively small amounts of seed being produced. In contrast, legitimate pollinations were highly fertile and all pollinations resulted in capsules and seed (Table 4).

*Population structure.* Morph frequencies within populations of *T. hermannioides* are isoplethic (equal proportions) and the pooled and heterogeneity G-statistics indicate the absence of heterogeneity among populations (Table 5). Presumably, this equilibrium is maintained by disassortative mating between the floral morphs as a result of the strong diallelic incompatibility system.

*Crossability with Turnera ulmifolia.* Observations of meiosis in plants of *T. hermannioides* from populations 1 and 4 indicated that

they were both diploid ( $2n = 2x = 10$ ) with regular bivalent formation. Results of the crossing program to investigate the biosystematic relationships between *T. hermannioides* and members of the *T. ulmifolia* complex are presented in Table 6. Crosses between the two taxa usually resulted in the initiation of capsules, however, fruits and developing seed commonly aborted after initial swelling. Barriers to crossability were particularly evident between diploid varieties of *T. ulmifolia* and *T. hermannioides*. Although approximately one-third of the pollinations that were conducted resulted in fruit, little seed was formed. Interestingly, significant numbers of seed were produced when *T. hermannioides* was used as the male parent in crosses with the two tet-

TABLE 5. Representation of floral morphs in four populations of *Turnera hermannioides* in Sergipé, northeastern Brazil. NS = not significant.

Population	No. of Long-styled Plants	No. of Short-styled Plants	$G^a$	df	$P$
1	60	57	0.08	1	NS
2	54	56	0.04	1	NS
3	60	53	0.43	1	NS
4	47	61	1.82	1	NS
Total	221	227	2.37	4	NS

<sup>a</sup>  $G$  (pooled) 0.08, df 1, NS;  $G$  (heterogeneity) 2.29, df 3, NS.

TABLE 6. Seed fertility in reciprocal crosses between *Turnera hermannioides* (population 4) and varieties of *Turnera ulmifolia*. All pollinations were performed under glasshouse conditions.

Varieties and Ploidy Level	No. of Pollinations	% Fruit Set	Mean Seed Set per Pollination ± s.d.
<b>A. <i>T. hermannioides</i> as ♀ parent</b>			
<i>surinamensis</i> (2x)	9	56	1.8 ± 2.6
<i>grandiflora</i> (2x)	12	25	0.4 ± 0.8
<i>intermedia</i> (2x)	8	25	0.8 ± 1.8
<i>intermedia</i> (4x)	6	83	4.3 ± 2.7
<i>elegans</i> (4x)	6	33	1.3 ± 1.8
<b>B. <i>T. hermannioides</i> as ♂ parent</b>			
<i>surinamensis</i> (2x)	10	30	1 ± 1.7
<i>grandiflora</i> (2x)	11	36	1.3 ± 2.1
<i>intermedia</i> (2x)	9	22	0.3 ± 0.7
<i>intermedia</i> (4x)	7	100	8.3 ± 3.6
<i>elegans</i> (4x)	7	71	13 ± 10.1



raploid varieties of *T. ulmifolia*. The reciprocal crosses were less productive. The crossing program demonstrates that *T. hermannioides* is, in large part, reproductively isolated from *T. ulmifolia* at the diploid level. Although  $F_1$  plants may be produced from crosses with tetraploid varieties, they would be triploid and probably sterile.

#### DISCUSSION

Apart from the absence of stigmatic polymorphisms, the distylous syndrome of *T. hermannioides* resembles those described in several other heterostylous families (reviewed in Ganders, 1979). The corolla morphology of distylous *Turnera* species is unusual for heteromorphic taxa because of the absence of a well-developed corolla tube. Flowers of *T. hermannioides* and *T. ulmifolia* are bowl-shaped and relatively unspecialized. Observations of visitors to flowers of both species indicated that they were visited by a wide range of insects, including *Apis mellifera*, *Xylocopa* sp., and various groups of solitary bees (see Barrett, 1978). It seems likely that with relatively generalized insect visitors and unspecialized flowers, considerable illegitimate pollination would occur, particularly in plants with large floral displays. The strongly developed self-incompatibility system in *T. hermannioides* and *T. ulmifolia* (Barrett, 1978) may thus be maintained to prevent self-fertilization.

There is little information on the geographic distribution of *T. hermannioides*. Based on our own field observations, as well as surveys of herbarium collections from Brazil, it would appear that the species occurs in scattered localities in the states of Sergipé, Bahia, and Minas Gerais. Diploid varieties of *T. ulmifolia* in Brazil also exhibit restricted and widely scattered distributions, frequently in association with local soil conditions. In striking contrast, polyploid vari-

eties of the *T. ulmifolia* complex are ruderal weeds with broad ecological tolerance and widespread distributions (Barrett, 1978; Bentley, 1979). Throughout northeastern Brazil, the tetraploid *T. ulmifolia* var. *elegans* is conspicuous as a weed of waste ground and roadsides, including those in which *T. hermannioides* occurs. Although *T. ulmifolia* var. *elegans* occurred intermingled with *T. hermannioides* in the four populations sampled, no putative hybrids were observed. While small numbers of seed may be produced from crosses between the two taxa all attempts by us to obtain viable seedlings have failed. The taxa are reproductively isolated from one another.

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